

Pharmacological Evaluation and Anti-Mycobacterial Potential of AYUSH-Based Bioactive Compounds against *Mycobacterium avium* subspecies *paratuberculosis*

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Abstract: A chronic granulomatous disease affecting domestic livestock and humans alike, Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). We need new ways of treating infections because immunizations aren't working and antibiotic resistance is on the rise. An AYUSH-based compound made from *Ocimum sanctum* and *Solanum xanthocarpum* was tested for its anti-mycobacterial activity in the present study. Phytochemical screening, the Resazurin microtiter assay (REMA), and molecular docking studies targeting the Dephospho-Coenzyme A kinase (DPCCK) protein of MAP were used to investigate bioactive substances, such as solasodine and ursolic acid. Ursolic acid demonstrated superior inhibitory activity with MIC₅₀ value of 12 µg/mL, while solasodine exhibited MIC₅₀ of 60 µg/mL. Molecular docking analysis showed strong ligand–receptor interaction comparable to rifampicin. In vivo immunomodulatory studies revealed enhanced cytokine regulation and splenocyte proliferation in treated rats. Toxicity evaluation demonstrated no significant biochemical or hematological abnormalities. The findings indicate that AYUSH-based bioactive compounds may serve as promising therapeutic candidates against paraTB infection.

Keywords: Johne's Disease, MAP, AYUSH, *Ocimum Sanctum*, *Solanum Xanthocarpum*, Ursolic Acid, Solasodine.

1. Introduction

Johne's disease (JD) is a chronic infectious disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), primarily affecting ruminants such as cattle, sheep, goats, and buffaloes [1]. The disease causes progressive weight loss, chronic diarrhea, malabsorption syndrome, and severe economic losses in livestock industries worldwide [2]. MAP infection has also been implicated in several autoimmune and inflammatory disorders in humans, including Crohn's disease, ulcerative colitis, rheumatoid arthritis, and type-1 diabetes [3].

The increasing prevalence of MAP infection, along with its resistance to conventional anti-tubercular therapy, has become a major public health concern [4]. Existing therapies involve prolonged multidrug regimens, which often result in toxicity and emergence of resistant strains [5]. Furthermore, currently available vaccines provide limited protection and interfere with diagnostic procedures [6]. Hence, there is an urgent need for safe, economical, and effective alternative therapeutics.

Medicinal plants have been widely explored in traditional Indian medicine systems for their antimicrobial and immunomodulatory properties [7]. *Ocimum sanctum* (Tulsi) and *Solanum xanthocarpum* (Kantakari) possess several pharmacologically active phytoconstituents such as ursolic acid, solasodine, eugenol, and flavonoids [8]. Previous studies have demonstrated their antibacterial, anti-inflammatory, antioxidant, and anti-mycobacterial activities [9].



The present study aimed to investigate the anti-MAP potential of bioactive compounds derived from *O. sanctum* and *S. xanthocarpum* using in vitro, in vivo, and in silico approaches. Special emphasis was placed on the inhibitory interaction of ursolic acid and solasodine with MAP-DPCK protein, an essential enzyme involved in coenzyme-A biosynthesis.

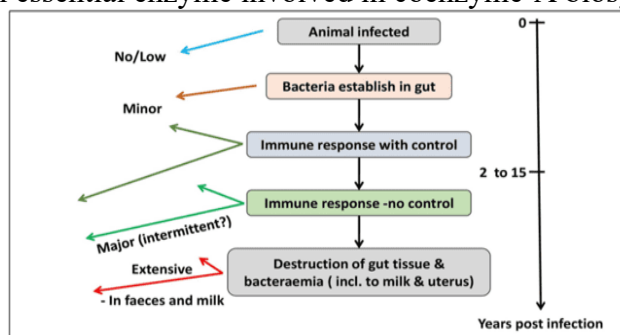


Figure 1: Pathogenesis of MAP infection in cattle.

2. Materials and Methods

Fresh plant materials of *Ocimum sanctum* and *Solanum xanthocarpum* were collected and authenticated. Hydroalcoholic and aqueous extracts were prepared using Soxhlet extraction. Preliminary phytochemical screening was performed to identify alkaloids, flavonoids, terpenoids, phenolics, and glycosides [10].

The anti-mycobacterial activity was evaluated using the Resazurin Microtitre Assay (REMA) against native MAP strain “S5.” The control group received the standard anti-tubercular medication regimen, which included rifampicin, clarithromycin, and ofloxacin [11]. Both solasodine and ursolic acid had their MIC50 values calculated.

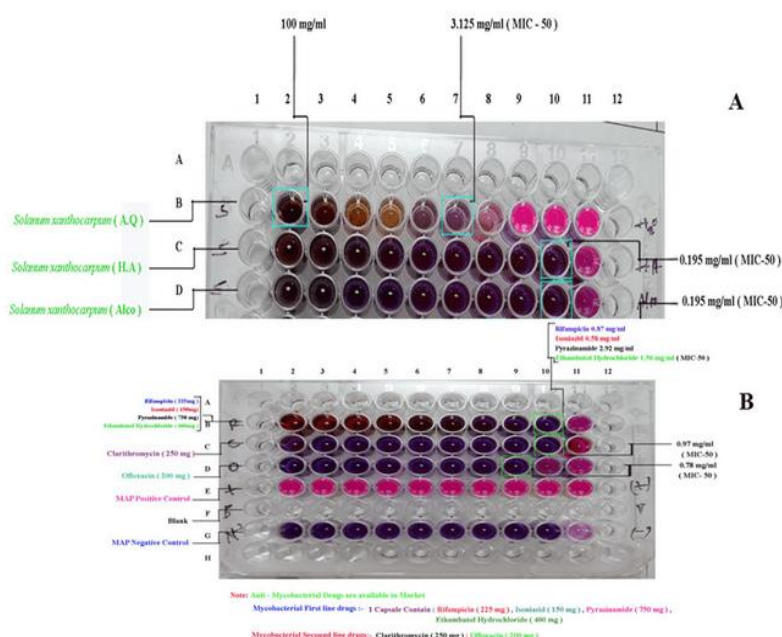


Figure 2: A test known as REMA. (A) The effects of *Solanum xanthocarpum* Schrad. & Wendl.'s A.Q. (aqueous), H.A. (hydro-alcoholic), and Alco (alcoholic) extracts in vitro; (B) The effects of 225 mg of Rifampicin, 150 mg of Isoniazid, 750 mg of Pyrazinamide, 400 mg of Ethambutol hydrochloride, and 200 mg of Clarithromycin and 200 mg of Ofloxacin on the in vitro tests.

For molecular docking studies, the MAP Dephospho-Coenzyme A kinase (DPCK) protein was selected as the target receptor. Ligand structures were retrieved from PubChem and

docking simulations were performed using AutoDock software [12]. Binding affinity, hydrogen bond interaction, and docking score were analyzed.

In vivo immunomodulatory studies were carried out using Wistar rats divided into different treatment groups. Splenocyte proliferation assay and cytokine expression analysis (IL-2, IFN- γ , TNF- α , IL-4, IL-6, and IL-10) were conducted using ELISA and qRT-PCR methods [13].

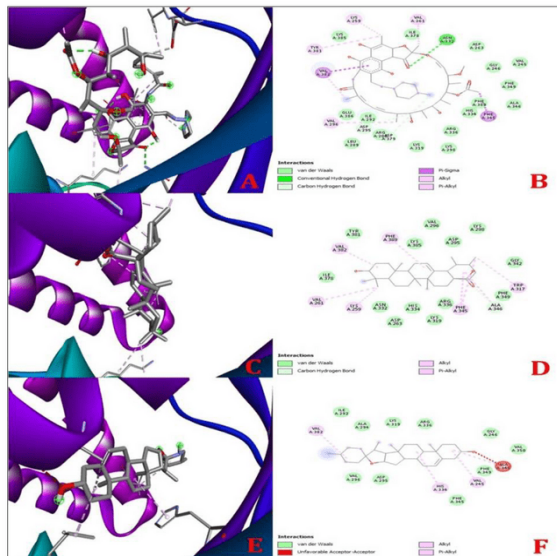


Figure 3: Illustrations of the optimal receptor-ligand interactions with respect to the MAP Dephospho-Coenzyme in three dimensions (left) and two dimensions (right).

In fig 1 & 2, Interaction between the DPCCK protein (C,D) and Rifampicin (control) (A, B). The ursolic acid-DPCCK protein interaction (E, F). Analysis of solasodine-DPCCK protein interaction. There are colored spheres that represent different types of residues in the target. Green spheres represent conventional hydrogen bonds, light green spheres represent carbon-hydrogen bonds, pink spheres represent pi-sigma bonds, light pink spheres represent alkyl and pi-alkyl bonds, and red spheres represent unfavorable acceptors. To help identify which ligands are compatible with the closest atom, the protein pocket uses color-coded lines. The space between the lines shows how the protein pocket opens. The 3D simulation makes use of both the stick model and the solid ribbon model to depict the ligands and proteins, respectively..

Acute oral toxicity studies were performed according to OECD guidelines. Biochemical, hematological, and histopathological parameters were evaluated after administration of synergistic AYUSH-based formulation (SABF) at doses ranging from 5–2000 mg/kg body weight.

3. Results

The study's ligand and receptor interactions are depicted in the figure above using ball-and-stick models. Pi-cations are displayed in green, alkyl bonds in blue, and hydrogen interactions in yellow. Using ZINC Pharmer 2022, we investigated the ligand-pharmacophore interaction's lack of efficacy.

Phytochemical analysis confirmed the presence of alkaloids, flavonoids, terpenoids, tannins, phenolics, and glycosides in both plant extracts. Ursolic acid and solasodine were identified as the major bioactive compounds responsible for anti-MAP activity [14].

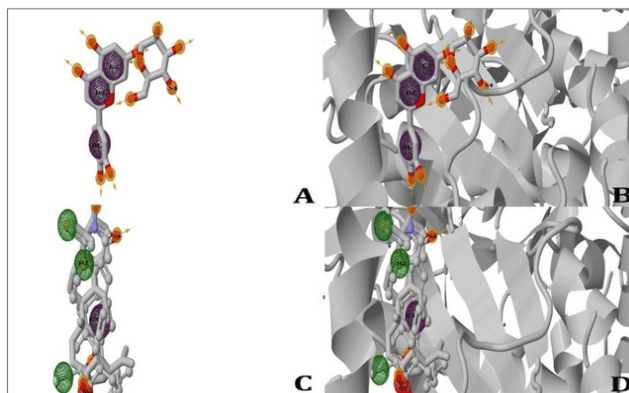


Figure 3: A three-dimensional representation of the pharmacophore study that was conducted to create the ligands that inhibit the MAP Dephospho-Coenzyme A kinase (DPCK) protein; Liquid solasodine (A) on the left; (B) Pharmacophore solasodine (right); (C) The ligand for ursolic acid (left), (D) The pharmacophore of ursolic acid (right).

The REMA assay demonstrated significant inhibition of MAP growth by ursolic acid and solasodine. Ursolic acid showed a MIC₅₀ value of 12 µg/mL, whereas solasodine exhibited MIC₅₀ of 60 µg/mL. Rifampicin showed higher activity but the phytoconstituents displayed promising inhibitory potential with reduced toxicity [15].

Molecular docking analysis revealed strong binding affinity between the ligands and MAP-DPCK protein. Ursolic acid exhibited superior docking interaction involving hydrogen bonding and hydrophobic interactions with active site residues. Docking scores indicated comparable interaction stability with standard anti-tubercular drugs [16].

In vivo studies demonstrated enhanced splenocyte proliferation and improved cytokine regulation in SABF-treated groups. Increased expression of IL-2 and IFN-γ suggested activation of Th1-mediated immunity, whereas reduced TNF-α levels indicated anti-inflammatory effects. The formulation significantly reduced bacterial load in intestinal tissues.

Acute toxicity studies showed no mortality or significant changes in body weight, organ indices, liver function, renal function, or hematological parameters. Histopathological examination confirmed absence of tissue damage, indicating safety of the formulation.

4. Discussion

The present study demonstrates the therapeutic potential of AYUSH-based phytoconstituents against MAP infection. Ursolic acid and solasodine exhibited substantial anti-mycobacterial activity supported by both in vitro and in silico analyses. The inhibitory action against DPCK protein suggests disruption of coenzyme-A biosynthesis, an essential pathway for MAP survival [17].

The immunomodulatory activity observed in splenocyte proliferation and cytokine expression studies indicates enhancement of host immune response. Elevated IFN-γ and IL-2 levels suggest activation of macrophage-mediated immunity, which is crucial for controlling intracellular mycobacterial infections [18].

The antioxidant and anti-inflammatory properties of the formulation may further contribute to reduction of chronic inflammation associated with paraTB infection. Similar findings have been reported for ursolic acid in tuberculosis and inflammatory disorders [19].

The absence of toxicity in treated animals highlights the safety profile of the synergistic formulation. Herbal therapeutics offer advantages such as lower toxicity, reduced resistance development, and better patient compliance compared to conventional antibiotics [20].

5. Conclusion

The AYUSH-based formulation developed from *Ocimum sanctum* and *Solanum xanthocarpum* demonstrated promising anti-mycobacterial, immunomodulatory, and anti-inflammatory activities against MAP infection. Ursolic acid and solasodine emerged as potent bioactive compounds with significant inhibitory action against MAP-DPCK protein. The synergistic formulation exhibited excellent safety profile and therapeutic efficacy in experimental models. The findings suggest that these phytoconstituents may serve as potential candidates for development of novel herbal therapeutics against paraTB and related mycobacterial diseases

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